Seasonal variation of concentration ratios for ICRP's Reference Animal and Plants in terrestrial Mediterranean ecosystems

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Abstract

The International Commission on Radiological Protection (ICRP) have proposed a system for the radiological protection of the environment based on the use of Reference Animals and Plants (RAPs).Seasonal variation may be important in all climatic types.In the case of Mediterranean ecosystem, seasonal variations may influence the transfer of elements in a different way than in temperate ecosystems. This is because the strong variation in temperature and precipitation can significantly alter the availability of nutrients in the ecosystem. In the present work, variation of whole organism concentration ratios, CR_{wo}, was determined for several stable elements (I, Li, Be, B, Na, Mg, Al, P, S, K. Ca, Ti, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Se, Rb, Sr, Mo, Ag, Cd, Cs, Ba, Tl, Pb and U) in selected terrestrial RAPs in two different Mediterranean ecosystems: a pinewood and a dehesa (grassland with disperse tree cover). For this paper the RAPs considered in the pinewood ecosystem were Pine Tree and Wild Grass; whereas in the dehesa ecosystem those considered were Deer, Rat and Wild Grass. Seasonal variation was observed to be likely site-dependent, and depended on the element-RAP combination.

Introduction

The ICRP have proposed a system for the radiological protection of the environment based on the concept of Reference Animals and Plants (RAPs) (ICRP 2008).There are different models in the literature to assess the dose rate to RAPs. Most of them, such as ERICA (Brown et al., 2016), RESRAD-BIOTA (USDoE, 2002), R&D128/SP1a (Copplestone et al., 2001; 2003) use a quasi-equilibrium approach to estimate the activity concentration in organisms and consequently their internal dose rate.Concentration ratios, CR_{wo}, are used in such models (Beresford et al. 2008) to predict activity concentrations in wildlife assuming that there is equilibrium between the whole organism (RAP) and the appropriate medium (e.g. soil in the case of terrestrial ecosystems). There is a lack of CR_{wo} data (ICRP, 2009) for many element-RAP combinations. CR_{wo} values are also likely to be highly site specific which contributes to the large variation observed within the

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available data,much of which originates from Europe andNorth America, mainly for temperate and artic ecosystems (Howard et al., 2013). As the CR_{wo} values are defined for quasi-equilibrium approach, it is assumed to be approximately constant throughout the year. However, seasonal variation of radionuclide transfer has been previously reported with an increase in radionuclide uptake from November to February for Mediterranean grazing-land ecosystems (Baeza et al., 2001). The alternation between hot dry seasons (mainly summer) and cold wet seasons (mainly winter) reflectsvariation innutrient availability in each season.Seasonal variation of transfer and element concentration inbees in Spain have also been reported (Gutiérrez et al., 2015). This seasonal pattern contrast to that observed in temperate ecosystems following the Chernobyl accident where radiocaesium concentrations in grazing animals and herbaceous vegetation was highest during summer months (see Beresford et al. 1996).

The goal of this study is to analyze the seasonal variation of CR_{wo} values for some terrestrial RAPs (Rat, Deer, Wild Grass and Pine Tree) collected in Mediterranean ecosystems for 32 elements (Ag, Al, As, Ba, Be, Ca, Cd, Co, Cr, Cs, Cu, Fe, I, K, Li, Mg, Mn, Mo, Na, Ni, P, Pb, Rb, Sr, Ti, Tl, U, V and Zn). The main sampling site was a Dehesa, which is a typical Mediterranean semi-natural grassland with disperse tree cover, mainly holm oaks. As there was no pine tree at this location, a Pinewood located in the vicinity was also selected. Pine Tree wood and Wild Grass were collected from this second site.

Materials and Methods

Sampling sites

Two locations were selected for sampling terrestrial RAPs in the province of Cáceres, western Spain, in the surroundings of Monfragüe National Park: a dehesa and a pinewood (Figure 1) (Guillén et al., 2016). The climate is dry sub-humid (Csa in Köppen classification), with annual average temperature of 16ºC and hot summer. The Dehesa extends over more than 4,600 ha.It serves as hunting reserve, mainly for red deer (*Cervuselaphus*) and wild boar (*Susscrofa*). Soil texture was silt-loam with a pH 6.5 at the Dehesa. As no pine treeswere present in the selected Dehesa, a Pinewood located about 16 km from Dehesa sitewas selected. It isa natural pinewood with no management. Wild grass and pine tree were sampled at this location. The texture of Pinewood site soil was loamy-sand with a pH 5.2.

Table 1 lists the representative species of RAPs sampled at the Dehesa and Pinewood sites. Rat, Deer, Wild Grass and Pine Tree were collected in different seasons (summer, autumn, winter and spring) during 2014/15. No Deer was sampled in spring, because it was close season.Individuals for vertebrate animals were skinned and different tissues were separated: muscle, bone, liver, kidney and thyroid. In summer no Deer thyroid was possible to collect, and therefore data for summer are not shown. Wild Grass collected in summer was very dry, with a dry content of about 77 % and higher. As the Rat thyroid gland was too small to isolate it directly, the area of tissue around it was sampled. Prior to analysis, biota samples were freeze-dried and stored in a desicator, ground using a nitrogen mill and then acid digested.

Soil samples(0 – 10 cm) were collected in the Dehesa and Pinewood at the same time as Wild Grass and Pine Tree sampling. For each season at each site 6 soil samples were collected from a 500 m^2 area and combined to form a composite sample for subsequent analysis. Samples were sieved, and fractions greater than 2 mm were discarded. Soil samples were then homogenized and oven dried (c. 60^oC).

ICP-MS analysis.

Soil digestion (about 0.2 g) was undertaken by adding concentrated HF, HNO_3 and $HClO_4$ (2.5:2:1 mL), and heating to 160°C overnight. Plant and animal tissues were acid digested with microwave oven at 140 ºC for 20 min. with: a) 6 mL *Primar* grade HNO_3 for plants, or b) HNO_3, MilliQ ultrapure water and 30% v/v H_2O_2 (3:2:2 mL) for animal tissues. Alkaline extraction with tetramethylammonium hydroxide (TMAH) was used to determine iodine content in the samples. Iodine analyses were carried out in thyroids samples and if enough mass was available (greater than 0.3 g dry matter (DM)) in the other sample types. For soils, aliquots of 1 g were weighed into polypropylene tubes and 10 mL of 10% TMAH were added. The soil suspensions were heated at 90°C for 24 h, and then centrifuged at 3500 rpm for 30 min.

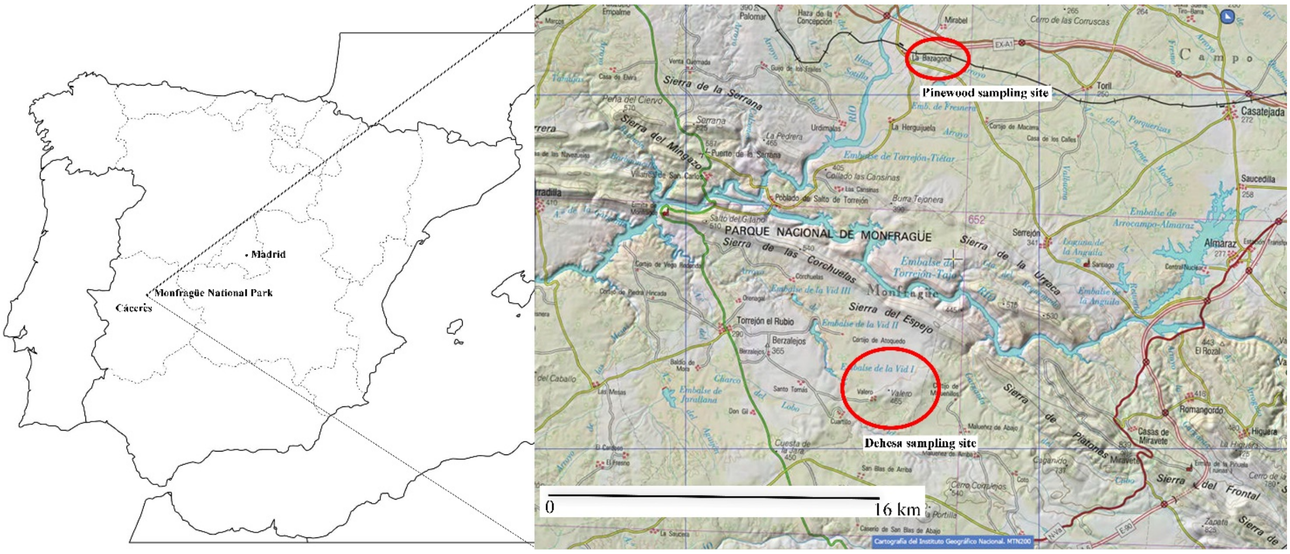

Figure 1.*General location of the Dehesa and Pinewood sampling sites.*

RAP	Family	Family/Species sampled	Sampling Site
Rat	*Muridae*	*Apodemussylvaticus*	Dehesa
Deer	*Cervidae*	*Cervuselaphus*	
Wild Grass	*Poceae*	*Briza minor*	Dehesa and Pinewood
Pine Tree	*Pinaceae*	*Pinuspinaster*	Pinewood

Table 1.*Representative species of terrestrial Reference Animals and Plants sampled from the Dehesa and Pinewood sites in different seasons (summer, autumn, winter and spring).*

Multi-element analysis of diluted solutions was undertaken by ICP-MS. Sample processing was undertaken using Qtegra™ software (Thermo-Fisher Scientific) utilizing external cross-calibration between pulse-counting and analogue detector modes when required.Iodine analysis was undertaken separately, using a 1% TMAH (tetramethylammonium hydroxide) matrix for standards and samples.

Detection limits were calculated as 3 times the standard deviation of the reagent blanks for each extraction form and sample type. Blank samples and Certified Reference Materials (CRM) NIST SRM 2711a Montana soil, NIST 1573a Tomato Leaves, NIST 1577c Bovine Liver were digested and prepared in a similar manner to check the accuracy and precision of the digestion and analysis methods.

Results and Discussion

The whole-body concentrations for Rat and Deer were calculated assuming that the tissues analyzed (thyroid, liver, kidney, meat and bone) represented the whole animal (an approach taken by Barnett et al. (2014) in a similar study). For Deer, fresh mass percentages of the whole-body for each tissue were assumed to be the same as roe deer collected in the UK (Barnett et al., 2014).

CR_{wo} is defined as the ratio between the equilibrium activity concentration of a radionuclide in an organism and the corresponding medium (ICRP 2009). In the existing models and data compilations CR_{wo} values are present by element assuming the same value for all isotopes (of that element) including stable isotopes (eq. 1) (Copplestone et al. 2013) so here CR_{wo} is defined as:

$$CR_{wo} = \frac{Concentration\ element\ X\ in\ whole\ body\ RAP\ (mg/kg\ FM)}{Concentration\ element\ X\ in\ soil\ (mg/kg\ DM)} \quad (1)$$

For comparative purposes, a selection of alkali (K, Rb, and Cs), alkaline earth (Ca, Sr and Ba), heavy metal (Pb) elements, and I and P have been used. Figure 2 shows the CR_{wo} variation for Rat in the period summer 2014 - winter 2015.A maximum in autumn can be observed for P, Rb and Pb within the same order of magnitude, and for Ca, Sr, Ba and Cs. Iodine concentrations were under detection limits in autumn, but the CR_{wo} in winter and spring were about one order of magnitude higher than the detection limits. The I CR_{wo} values should be considered as provisional at the moment, as they require some additional verification.

Figure 3 shows the CR_{wo} seasonal variation for Deer. It showed a lower degree of seasonal variation compared to Rat. A slight increase in autumn can be observed in P, Ca, Sr, Ba, which may be related to the antler generation;antlers are formed by bone tissue, shed annually in spring and regenerated again at the beginning of autumn. Antlers have been reported to bioaccumulate ^{90}Sr, and stable alkaline earth elements (Ca, Mg and stable Sr) (Baeza et al., 2011).Antler generation may be responsible for the autumn maximum in CR values for these elements in Deer, due to the need for nutrients required for their growth. This need was also reported in the traslocation of these elements from bone to antlers (Baxter et al., 1999). The CR_{wo} for Pb presented a variation about one order of magnitude, with a minimum in autumn. However, the Cs CR_{wo} values in autumn and winter increased compared to those in summer. The K CR_{wo} presented minimal variations, about 7 % variation over the mean value, due to its homeostatic behaviour.

Figure 4 shows the seasonal variation of CR_{wo} for Wild Grass at the Dehesa and Pinewood sampling sites. It can be observed that there is no similar trend for all radionuclides, suggesting that seasonal variations may be related to nutrient and water seasonal variations in each site. In the Dehesa site, a maximum in autumn can be observed for most elements, with variations of 1-2 orders of magnitude across the year. In the Pinewood site, this trend (maximum value in autumn) was only observed for P and Ca, which may be related to the nutrient availability. The rest of elements showed a decreasing trend in Pinewood site, with maximum values in summer.

Figure 5 shows the seasonal variation of CR_{wo} for Pine Tree. It presented the smallest seasonal variability of the RAPs analyzed, the variation was kept in the same order of magnitude. The P CR_{wo} values presented the same variation as the other RAPs analyzed, a maximum value in autumn. The Cs CR_{wo} presented a variation about one order of magnitude, but it was under detection limit in summer and spring.

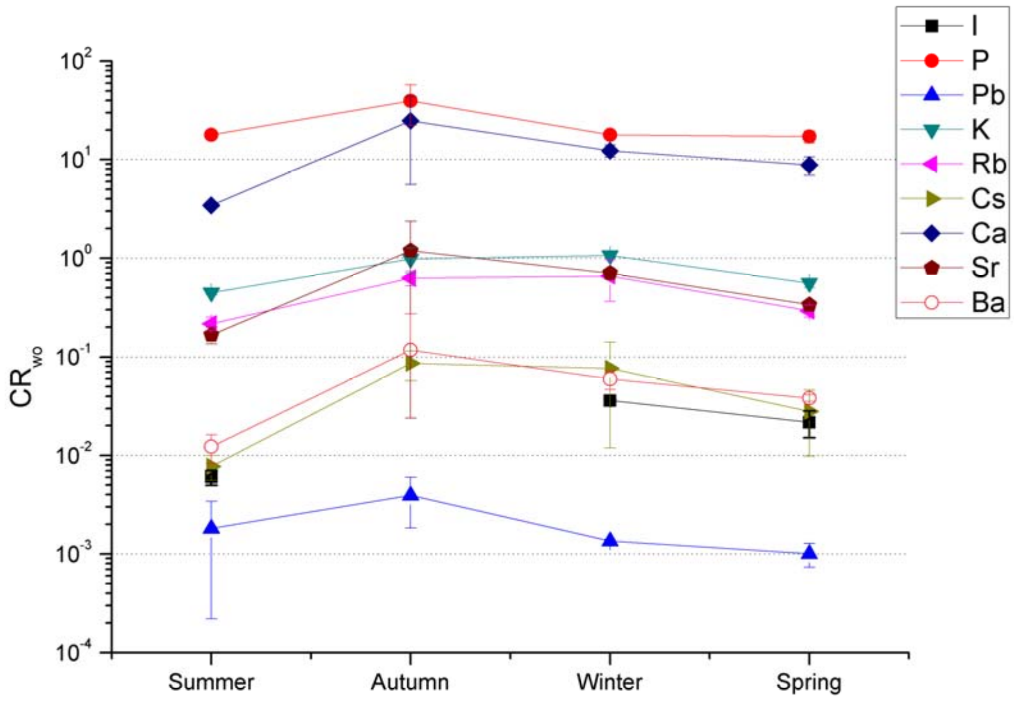

Figure 2.*Seasonal variation of CR_{wo} values for I, P, Pb, K, Rb, Cs, Ca, Sr and Ba for Rat.*

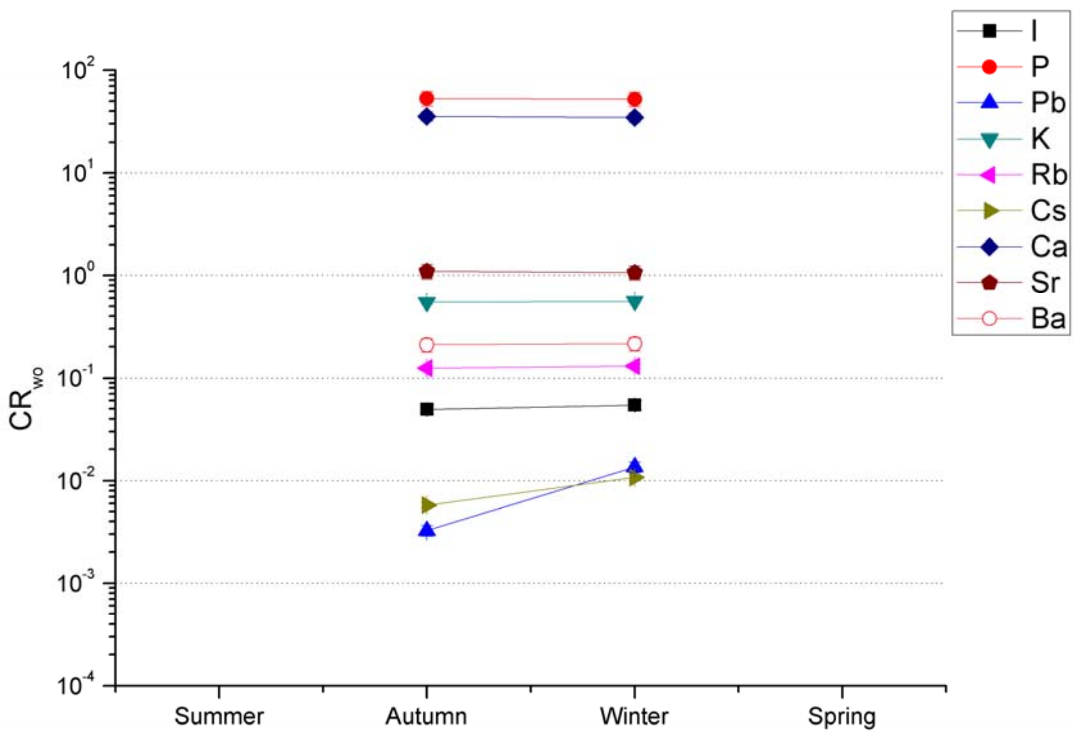

Figure 3.*Seasonal variation of CR_{wo} values for I, P, Pb, K, Rb, Cs, Ca, Sr and Ba for Deer.*

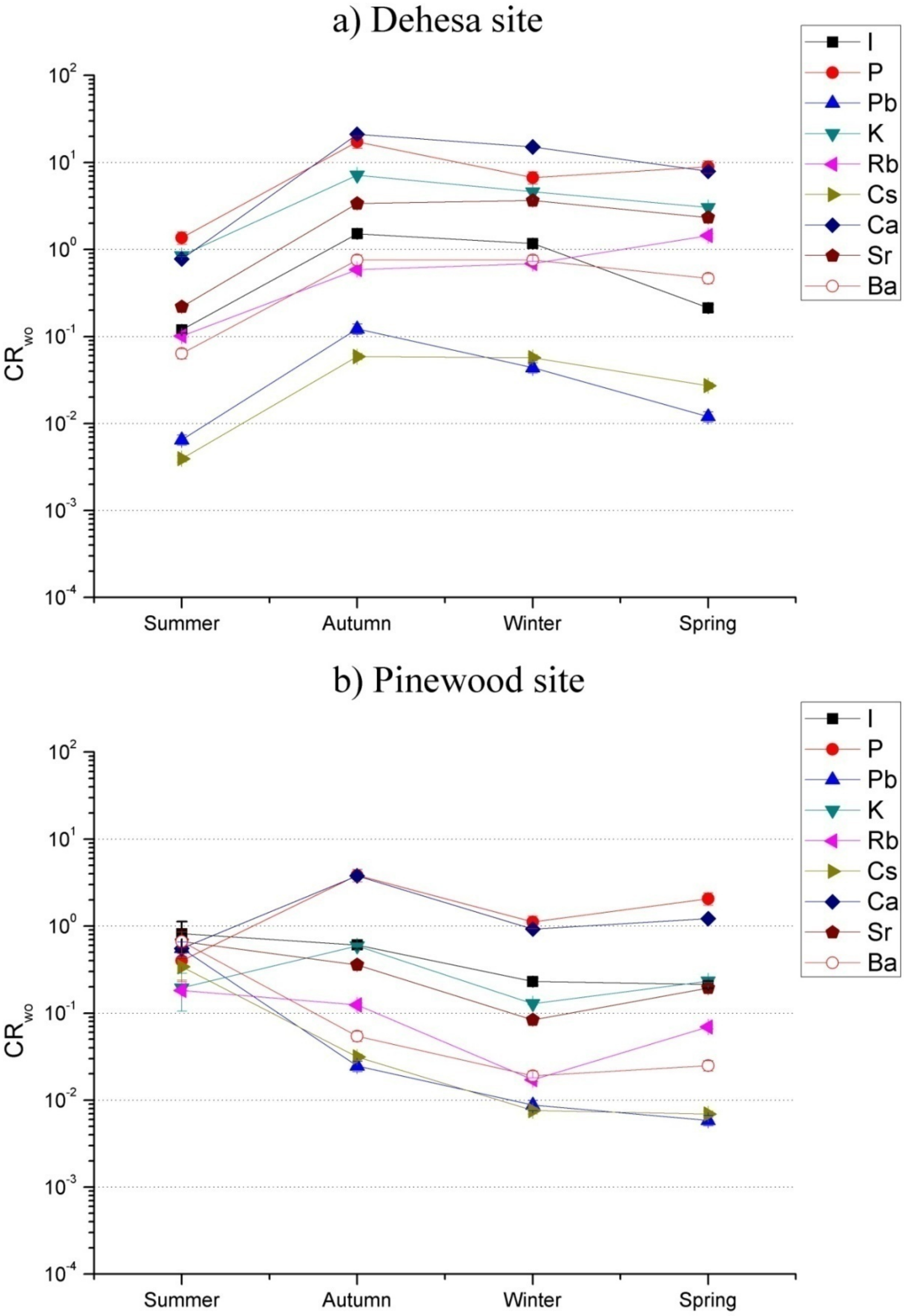

Figure 4.*Seasonal variation of CR_{wo} values for I, P, Pb, K, Rb, Cs, Ca, Sr and Ba for Wild Grass sampled in a) Dehesa site and b) Pinewood site.*

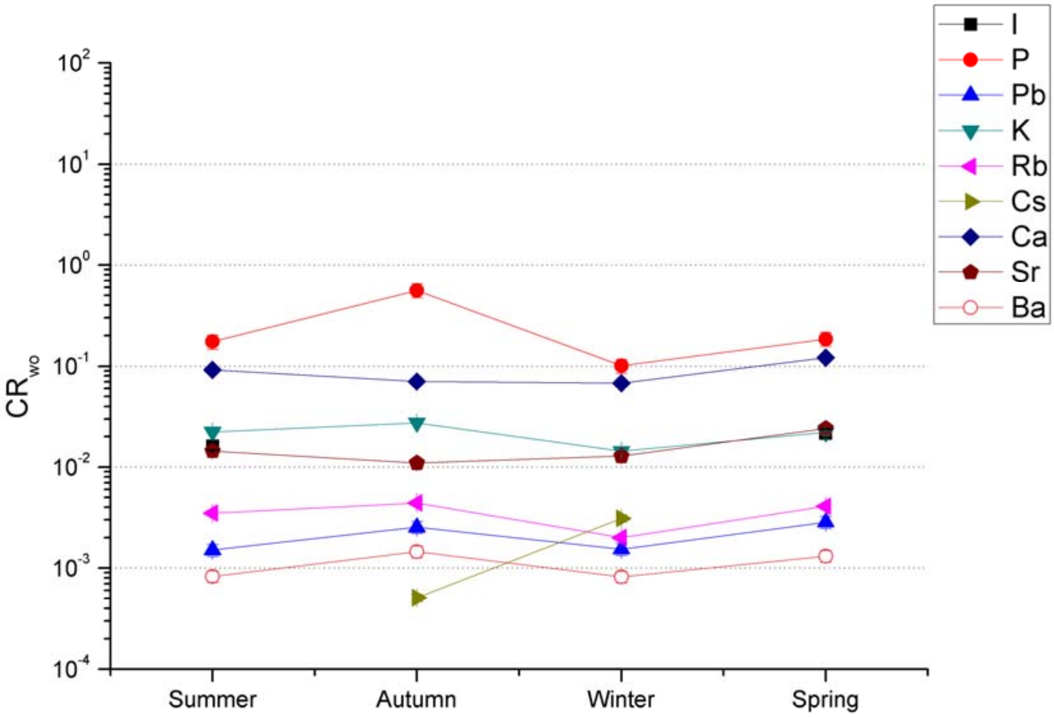

Figure 5.*Seasonal variation of CR_{wo} values for I, P, Pb, K, Rb, Cs, Ca, Sr and Ba for Pine Tree.*

Conclusions

The seasonal variation of CR_{wo} values for different elements (I P, Pb, K, Rb, Cs, Ca, Sr and Ba) was analyzed for different RAPs (Rat, Deer, Wild Grass and Pine Tree) in two Mediterranean ecosystems (Dehesa and Pinewood).

- Seasonal variations up to 1 - 2 orders of magnitude were observed.
- This variation was not the same for all elements and RAPs.
- Seasonal variation is likely to be site dependent, as for most elements the seasonal variation in Wild Grass was different in the Dehesa and Pinewood ecosystems, maybe related to nutrient and water availability.
- However, the seasonal variation for P was the same for all RAPs and sites, showing a maximum value in autumn.A similar trend was observed for Ca in all RAPs, except for Pine Tree.

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